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13. ABSTRACT (Maximum 200 words)

This final progress report presents the research work achieved under this contract on the nucleoside based lipids. Most of the molecules synthesized have shown remarkable physico-chemical properties. The supramolecular systems obtained are promising in many aspects and could lead to material suitable for biomacromolecules transport (DNA, RNA, siRNA..) and/or preparation of Hybrid Lanthanides and actinides microspheres. A summary of the most important results is presented.

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FINAL PROGRESS REPORT

Amphiphiles for DNA Supramolecular Assemblies
Grant # DAAD-19-02-1-0386

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Amphiphiles for DNA Supramolecular Assemblies

Abstract. This final progress report presents the research work achieved under the contract DAAD-19-02-1-0386. Most of the nucleoside based amphiphiles synthesized have shown remarkable physico-chemical properties. The supramolecular systems obtained are promising in many aspects and could lead to material suitable for biomacromolecules transport (DNA, RNA, siRNA..) and/or preparation of Hybrid Lanthanides and actinides microspheres. A summary of the most important results is presented.

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(2) Statement of the problem studied

Supramolecular chemistry has and continues to have a significant impact on basic research and industry.[1] Examples of supramolecular assemblies that highlight the underlying principles are evident in numerous biological (e.g., lipids) and synthetic (e.g., nanofibers) systems.[2] These hierarchical organizations are, typically, a consequence of multiple noncovalent interactions. The critical need in designing new supramolecular systems is the development of new simple molecular structures allowing multiple intermolecular interactions. The use of molecular recognition (e.g., H-bonding, π-stacking, electrostatics, etc) and metal coordination is an efficient and powerful

approach to prepare assemblies having new architectures with tunable physico-chemical properties.

The research work developed under this contract is aimed at designing new bio-inspired amphiphile structures for multiple applications ranging from molecular building blocks for self-assemblies, to drug or biomolecule delivery systems. In order to take advantage of forces that hold nucleic acid helices together, (Watson-Crick/Hoogsteen hydrogen-bonding and base stacking) we have proposed new amphiphiles possessing a DNA recognition unit (e. g., nucleobase) and a hydrophobic segment (figure 1). Included in this goal are the systematic studies of self-assemblies in the presence or absence of nucleic acids. The supramolecular devices developed can be used as transfection reagents, carriers for bioactive molecules, or as nano-organized hybrid materials involving block-f elements such as actinide and lanthanide. In addition, these aggregates could catalyze chemical reactions in constrained environments (polymerization, hydrolysis, etc).

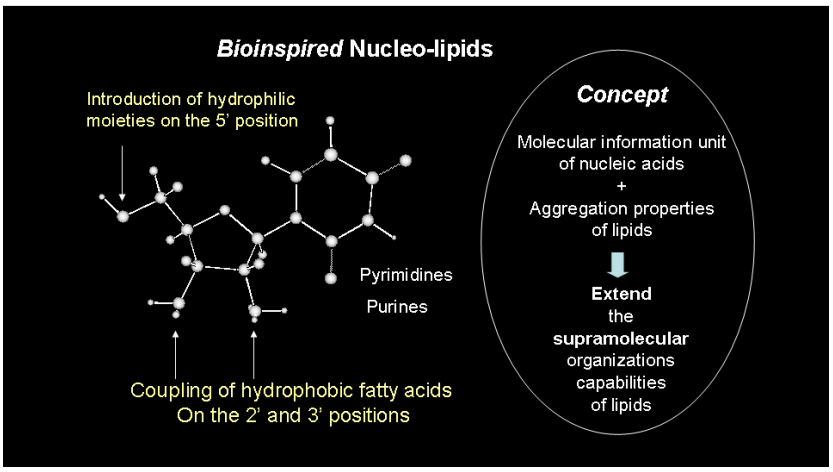

Figure 1. Design of nucleolipids.

(3) Summary of the most important results

Since 2002, our group has been designing novel amphiphilic structures derived from nucleobases. Our investigations linked to this research program entitled "Amphiphiles for DNA Supramolecular Assemblies", focused on the structure and self-organization relationships.[3] Specifically, how changes in the molecular structure affect the physio-chemical properties and assembly architecture. Thus, to better understand the chemical structural parameters that dictate self-assembly, the molecular structure of conventional lipids were modified *via* incorporation of additional molecular recognition features that can π-stack and hydrogen bond.

In our design, we use the ribose as a scaffold for the construction of the nucleolipid structures. This synthetic approach enables the preparation of a large variety of, non-ionic, anionic, cationic and zwitterionic, amphiphiles. Thanks to the ARO support we have synthesized and characterized a family of uridine and adenosine based amphiphiles. Several examples of nucleolipid structures including lipidic (I), fluorinated VII, ketals VIII) non-ionic (II, III, IV, V), anionic (IX) and cationic species (X, XI) are shown in figure 2.

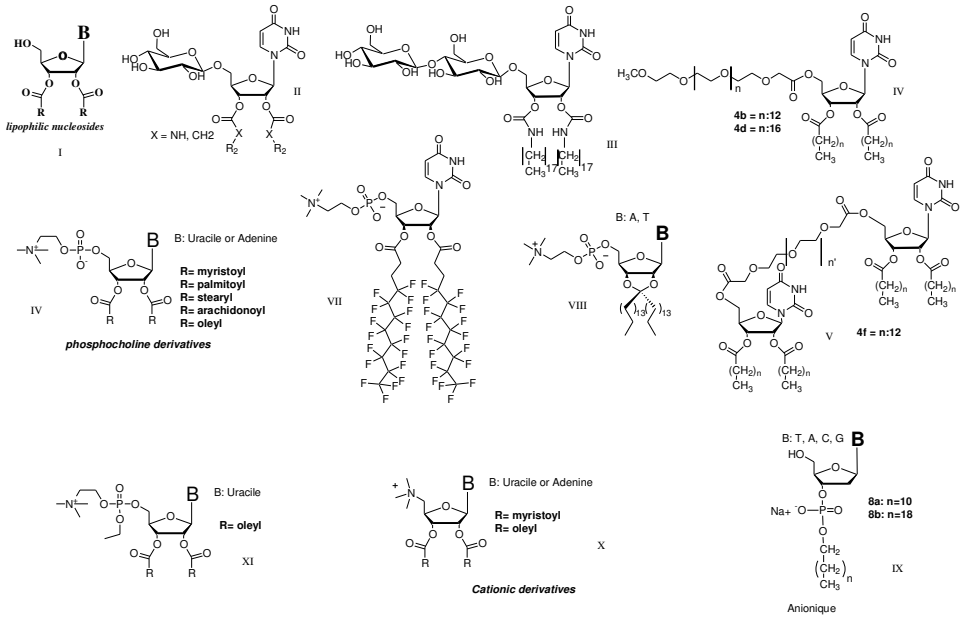

Figure 2. Examples of nucleoamphiphiles synthesized during the programme.

We discovered that these molecules posses unique properties such as the formation of aggregates including fibers, vesicles and micro spheres (figure 3). Additionally, they form hydrogels and organogels. The supramolecular systems obtained are promising in many aspects and could lead to new types of materials for transport of biomacromolecules (DNA, RNA, siRNA).[4]

a) Nucleoside based phosphocholine Amphiphiles [see publications #1 and #2]

Several nucleoside based phosphocholine Amphiphiles were prepared using simple synthetic strategies. Our investigations demonstrate that these molecules possess unique properties compared to their natural glycerol analogues. In aqueous solutions these compounds self-assemble into liposome-like bilayer structures for the “fluid” phase above the main phase transition temperature (Tm) and DNA-like helical fibers in the crystalline solid state below Tm. When the concentration of fibers is increased, a hydrogel forms with micron-sized cavities. These materials are thermoresponsive, and fibers or bilayers can be formed by simply changing the temperature. Of the nucleoside phosphocholine amphiphiles investigated, DPUPC exhibits ambidextrous gelation and forms gels in water and cyclohexane. The hydrogels are stable, and DNA can be entrapped with these structures. Such

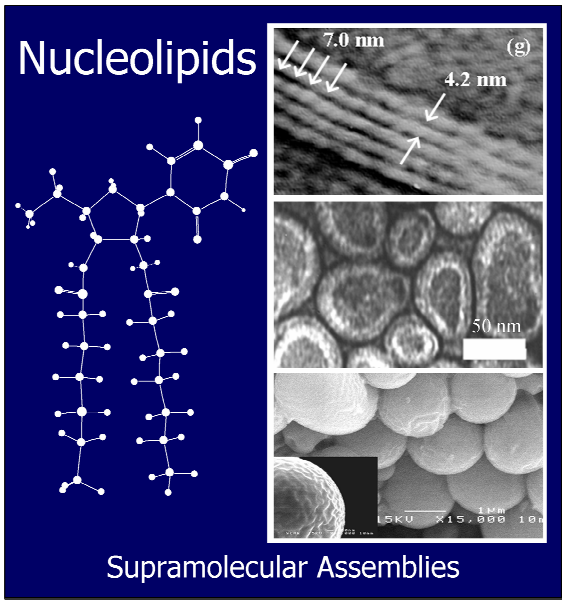

Figure 3. Left, nucleolipid structure. Right, TEM and SEM images showing examples of nucleolipid self-assemblies: nano-fibers, vesicles, and micro spheres respectively.

materials are likely to be of interest for medical and tissue engineering applications. The supramolecular assemblies prepared with nucleoside based phosphocholine Amphiphiles illustrate the importance of molecular structural complexity on amphiphile self-assembly.

b) Bio-inspired transfection reagents
[see publications # 3, # 4, # 5, and manuscript submitted #1]

Powerful transfection reagents derived from nucleolipids with high transfection efficiencies and poor cytotoxicities have been developed. Figure 4 shows an example of transfection results obtained with the nucleoside based lipid, *DEPC*. The diversity of nucleolipid supramolecular assemblies offers new transfection formats allowing a micro and nano-formulation of nucleic acids. Two promising candidates *DEPC* and *DOTAU* are currently under evaluation by leading biotechnology companies in the field of transfection.

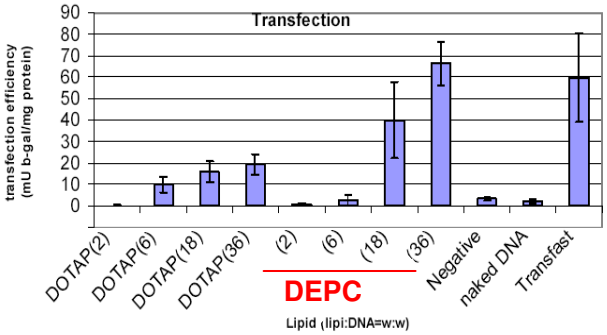

Figure 4. Transfection results of DEPC (CHO cells). DEPC is an efficient transfection agent greater than DOTAP and comparable to Transfast

c) Lanthanides and actinides Hybrid microspheres
[see manuscript submitted #2]

We are currently investigating the nucleolipid/block-f-elements (actinide, lanthanide) interactions. Microspheres composed of nucleoside based amphiphiles and f-block elements were obtained spontaneously by hydration of the nucleoside based amphiphile at a temperature slightly higher than its T_m with an aqueous solution containing either actinide or lanthanide salts. The supramolecular structure of these microspheres was characterized by light microscopy, TEM, SEM, SAXS, 31P NMR, and FTIR. The data collected indicate that the formation of these objects is a consequence of the following concomitant stabilizing factors: i) hydrophobic interactions, ii) U-U base pairing and iii) phosphate/f-block element salt binding. The resulting hollow actinide

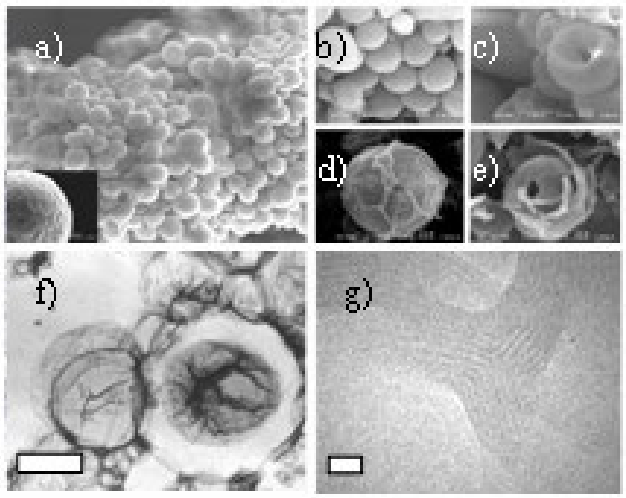

Figure 5. Typical SEM images of thorium (a), (b), (c), and Cerium (d) microspheres at different magnification. Inset (a): magnification of a thorium microsphere surface. (c) and (e) are images of respectively thorium and cerium destroyed microspheres showing their hollow stucture. (f) and (g) are typical TEM images of DSUPC-block f elements microspheres (1f bar = 3 µm, 1g bar = 20 nm).

or lanthanide microspheres possess hybrid nano structured shell.

A simple sonication procedure allows the preparation of individual actinide and/or lanthanide loaded microspheres, which can be used as micro-objects suspended in aqueous media.

Hybrid materials could provide new useful objects, which could easily find applications in the fields of neutron capture radio-therapy, nuclear fuel design and heavy metals (or radioactive nucleides) waste decontamination. Moreover, such hybrid materials could for example provide effective chelation therapy in response to internal or external human actinide contamination. Figure 5 shows scanning electron microscopy images of microcapsules containing Thorium or Cerium salts.

(4) Listing of all publications and technical reports supported under this grant or contract. Provide the list with the following breakout, and in standard format showing authors, title, journal, issue, and date.

(a) Papers published in peer-reviewed journals

1. Moreau, L.; Barthelemy, P.; El Maataoui, M.; Grinstaff, M. W., Supramolecular assemblies of nucleoside phosphocholine amphiphiles. *J. Am. Chem. Soc.* 2004, 126, (24), 7533-9.

2. Moreau, L.; Grinstaff, M. W.; Barthelemy, P., Vesicle formation from a synthetic adenosine based lipid. *Tetrahedron Lett.* 2005, 46, (10), 1593-1596.

3. Moreau, L.; Barthélémy, P.; Li, Y.; Luo, D.; Prata, C. A. H.; Grinstaff, M. W., Nucleoside Phosphocholine Amphiphile for in vitro DNA transfection. *Molecular BioSystems* 2005, 1, 260-264.

4. Arigon, J.; Prata, C. A.; Grinstaff, M. W.; Barthelemy, P., Nucleic Acid complexing glycosyl nucleoside-based amphiphile. *Bioconjug. Chem.* 2005, 16, (4), 864-872.

5. Barthélémy, P.; Camplo, M., Functional Amphiphiles for Gene Delivery. *MRS Bulletin* 2005, 30, (9), 647.

6. Barthélémy, P.; Lee, S. J.; Grinstaff, M., W., Supramolecular assemblies with DNA. *Pure and Applied Chemistry* 2005, in press.

(b) Papers presented at meetings, but not published in conference proceedings

Invited lectures and Oral Communications

1. Arigon, J.; Prata, C. A. H.; Grinstaff, M. W.; Barthélémy, P., Neutral nucleoside-based amphiphile for DNA binding. In 40th IUPAC Congress, Beijing, China, 2005.

2. Moreau, L.; Arigon, J.; Campins, N.; Chabaud, P.; Camplo, M.; Lee, S. J.; Grinstaff, M. W.; Barthélémy, P., Supramolecular assemblies of nucleolipids. In DNA Supramolecular Assemblies, Avignon, France, 2004.

3. Barthélémy, P., Amphiphiles dérives de nucléosides. Seminar, CEA Saclay, Gif sur Yvette, France, 2004.

4. Barthélémy, P.; Moreau, L.; Arigon, J.; Campins, N.; Chabaud, P.; Camplo, M.; Grinstaff, M. W., Supramolecular Assemblies of Nucleoside-Based Amphiphiles. In Surfactants and Their Assemblies: Future Opportunities, Jackson Hole, Wyoming, USA, 2004.

5. Barthélémy, P., Design of self assemblies with bio-inspired amphiphiles. In Seminar, ETH Zurich, Switzerland, 2004.

Posters and Oral Communications by Graduate Students:

1. J. Arigon, J.M. Lacombe, M. W. Grinstaff, Ph. Barthélémy, « Synthèse de nucléoamphiphiles non-ioniques dérivés de sucres » Société Francaise de Chimie, "XVIeme Journée de la Chimie SFC PACA", 3 avril 2003, Marseille, France

2. L. Moreau, M. El Maatoui, M. W. Grinstaff, Ph. Barthélémy, « Synthèse et caractérisation de nucléosides amphiphiles dérivés de phosphatidylcholine » Société Francaise de Chimie, "XVIeme Journée de la Chimie SFC PACA", 3 avril 2003, Marseille, France

3. Y. Chaudier, L. Moreau, J. Arigon, Ph. Barthélémy, « Synthèse d'amphiphiles Géminis nucléosidiques » XVIeme Journée de la Chimie SFC PACA", 3 avril 2003, Marseille, France

4. Ph Barthélémy, Syed Nadeem, Benjamin W. Maynor, Mark W. Grinstaff , "Nucleo-amphiphiles for DNA supramolecular assemblies" 39th IUPAC Congress and 86th Conference of the Canadian Society for Chemistry, August 10-15, 2003 Ottawa Congress Centre in Ottawa, Canada

5. Ph Barthélémy, Carla Prata, Shaun F. Filocamo, Mark W. Grinstaff , "Neutral nucleoside amphiphiles for the condensation of DNA" 227th ACS National Meeting, Anaheim, CA, March 28-April 1, 2004, USA.

6. Louis Moreau, Ph Barthélémy, M. El Maatoui, Mark W. Grinstaff , "Hydrogels of DNA and nucleoside based phosphocholine amphiphiles" 227th ACS National Meeting, Anaheim, CA, March 28-April 1, 2004, USA.

7. Carla A. H. Prata, Yuxing Zhao, Philippe Barthélémy, Yougen Li, Dan Luo, Thomas J. McIntosh, Stephen J. Lee, and Mark W. Grinstaff "Charge-Reversal Amphiphiles for Gene Delivery." 227th American Chemical Society, Anaheim, CA, 2004. ACS-ABS-BIOT n°147.

8. Louis Moreau, Mark W. Grinstaff, M. El Maatoui, Ph Barthélémy, “Organisation supramoléculaire de nucléolipides synthétiques : membranes et helices” Journées Franco Italiennes de Chimie, 1 et 2 avril, 2004, Genova, Italie.

9. Jérome Arigon, Mark W. Grinstaff, Ph Barthélémy, “Synthèse de nucléoamphiphiles glycosylés” Journées Franco Italiennes de Chimie, 1 et 2 avril, 2004, Genova, Italie.

10. Pauline Chabaud, Michel Camplo, Guillaume Serin, Louis Moreau, Philippe Barthélémy, Mark W. Grinstaff “Cationic nucleolipids for transfection” DNA Supramolecular Assemblies Workshop, Avignon, 2004, France.

11. Jérôme Arigon, Carla Hernandez Prata, Mark W. Grinstaff and Philippe Barthélémy « Glycosylated nucleoamphiphiles » DNA Supramolecular Assemblies Workshop, Avignon, 2004, France.

12. Nathalie Campins, Louis Moreau, Mark W. Grinstaff, Philippe Barthélémy, “Phosphorylated nucleoamphiphiles” DNA Supramolecular Assemblies Workshop, Avignon, 2004, France.

13. Louis Moreau, Mark W. Grinstaff, Mohamed El Maataoui, Philippe Barthélémy, “Supramolecular Assemblies of Nucleoside Phosphocholine Amphiphiles” DNA Supramolecular Assemblies Workshop, Avignon, 2004, France.

14. Chad E. Immoos, Ph Barthélémy, Carla A. H. Prata, Shaun F. Filocamo, Benjamin W. Maynor, Stephen J. Lee, Mark W. Grinstaff , “Neutral nucleoside amphiphiles for DNA condensation and DNA-supramolecular assemblies” DNA Supramolecular Assemblies Workshop, Avignon, 2004, France.

15. Carla A. H. Prata, Yuxing Zhao, Philippe Barthélémy, Yougen Li, Dan Luo, Thomas J. McIntosh, Stephen J. Lee, and Mark W. Grinstaff “Charge-Reversal Amphiphiles for Gene Delivery” DNA Supramolecular Assemblies Workshop, Avignon, 2004, France.

16. Jérôme Arigon, Carla Hernandez Prata, Mark W. Grinstaff and Philippe Barthélémy « Glyco-nucleo-amphiphiles » « Journée Biologistes, Chimistes et Physiciens,...aux frontières du vivant » 13 décembre 2004, Marseille, France.

17. Nathalie Campins, Louis Moreau, Mark W. Grinstaff, Philippe Barthélémy, “Single chain nucleo-amphiphiles” «Journée Biologistes, Chimistes et Physiciens,...aux frontières du vivant » 13 décembre 2004, Marseille, France.

18. Louis Moreau, Mark W. Grinstaff, Mohamed El Maataoui, Philippe Barthélémy, “Nucleoside Phosphocholine Amphiphiles” « Journée Biologistes, Chimistes et Physiciens,...aux frontières du vivant » 13 décembre 2004, Marseille, France.

19. Louis Moreau, Philippe Barthélémy, Yougen Li, Dan Luo, Carla A. H. Prata and Mark W. Grinstaff. « Nucleoside Phosphocholine Amphiphile for in vitro DNA transfection » 229th American Chemical Society, San Diego, CA, March 13-17, 2005.

20. Jérôme Arigon, Carla Hernandez Prata, Mark W. Grinstaff and Philippe Barthélémy «DNA complexing glycosylated nucleoside based amphiphiles » « 1st International Symposium on Biomolecules and Related Compounds», Montpellier, France 20-25 /03/ 2005.

21. Nathalie Campins, Louis Moreau, Mark W. Grinstaff and Philippe Barthélémy, « Ribbon-like structures from phosphorylated nucleoamphiphiles » « 1st International Symposium on Biomolecules And Related Compounds», Montpellier, France, 20-25 /03/ 2005.

22. Louis Moreau, Mark W. Grinstaff, Mohamed El Maataoui, Philippe Barthélémy, "Supramolecular architecture from nucleoside phosphocholine amphiphiles" « 1st International Symposium on Biomolecules and Related Compounds», Montpellier, France, 20-25 /03/ 2005.

23. Jerome Arigon, Carla A. H. Prata and Mark W. Grinstaff and Philippe Barthélémy, "Neutral nucleoside-based amphiphile for DNA binding", 43rd IUPAC General Assembly and 40th IUPAC Congress, Beijing, China, August 13-21, 2005.

24. Nathalie Campins, Mark W. Grinstaff et Philippe Barthélémy, « Organisations en rubans d'amphiphiles dérivés de nucléotides», 18e Journée Régionale de la Chimie SFC-PACA. Université du Sud Toulon Var La Garde, France, le 7 avril 2005.

25. Louis Moreau, Philippe Barthélémy, Yougen Li, Dan Luo, Carla A. H. Prata et Mark W. Grinstaff. «Transfection in vitro à l'aide d'Amphiphile dérivés de Nucléoside», 18e Journée Régionale de la Chimie SFC-PACA. Université du Sud Toulon Var La Garde, France, le 7 avril 2005.

26. Jerome Arigon, Mark W. Grinstaff et Philippe Barthélémy, "Nucleoamphiphiles glycosylés comme agents de complexation d'acides nucleiques", 18e Journée Régionale de la Chimie SFC-PACA. Université du Sud Toulon Var La Garde, France, le 7 avril 2005.

27. Louis Moreau, Philippe Barthélémy, "Architectures supramoléculaires et nucléolipides", Journée de la Fédération de Chimie de Marseille, le 28 avril 2005.

(c) Manuscripts submitted, but not published

1. Pauline Chabaud, Michel Camplo, Dominique Payet, Guillaume Serin, Louis Moreau, Philippe Barthélémy, and Mark W. Grinstaff, "Cationic Nucleoside Lipids derived for Gene Delivery." submitted to *Bioconj. Chem.* 08-2005.

2. Louis Moreau, Fabio Ziarelli, Mark W. Grinstaff and Ph. Barthélémy, "Spontaneous Formation of Hollow Microspheres From f-Block Elements and Nucleoamphiphiles" submitted to *J. Am. Chem. Soc..,* 10-2005.

(d) Technical reports submitted to ARO

Barthélémy, P.; 9 technical interim reports (# 1 to # 9)

(5) List of all participating scientific personnel showing any advanced degrees earned by them while employed on the project

1. Jérome Arigon (PhD défense 07-08-2005)
2. Louis Moreau (PhD défense 12-16-2005)
3. Nathalie Campins

(6) Report of Inventions (by title only)

"New amphiphilic compounds, synthesis and applications in particular to the transfection". Ph. Barthélémy, M. Camplo, M. W. Grinstaff, L. Moreau, French patent. 03-2004, (n° INPI 0404554).

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[1] a) I.W. Hamley, *Angew. Chem. Int. Ed. Engl*. **2003**, *42*, 1692-1712; b) J.-M. Lehn, *Sciences* **2002**, *295*, 2400-2403.

[2] a) Z. R. Tian, J. Liu, J. A. Voigt, B. Mckenzie, H. Xu, *Angew. Chem.*, *Int. Ed.* **2003**, *42*, 414-417. b) H. Cölfen, S. Mann, *Angew. Chem.*, *Int. Ed.* **2003**, *42*, 2350-2365. c) M. C. T. Fyfe, J. F. Stoddart, *Acc. Chem. Res.* **1997**, *30*, 393-401.

[3] "Amphiphiles for DNA Supramolecular Assemblies" Proposal No. 44395-CH, Grant No. DAAD19-02-1-0386

[4] P. Barthélémy, M. Camplo, M. W. Grinstaff, L. Moreau, French patent. 03-2004, (n° INPI O4O4554).

(8) Appendixes

Publications 1-6, and Manuscripts submitted, but not published